containing 50 % shells and 50% seeds (by weight) and the other version containing 70% of shells and 30% of seeds (by weight). All animals 16 dogs and 16 horses were treated for a time period of three month, the dose was 0.3 g pr kg daily, and data was lumped together. The human studies also tested two different versions of the same shell and seed containing Rose-hip remedy: The daily dose of one version was 1.2 g seeds and a similar amount of shells (A version) and the daily dose of the version (B) was 0.2 g seeds combined with 1.8 g shells.
The human study was performed in two groups of middle aged patients with modest osteoarthritis. A seasonal variation is reported for CRP (higher during winter), and both groups started treatment late autumn. A number of 43 patients were given high dose seed version and 40 other patients were given corresponding placebo. Another group of 32 patients (B group) were given the low seed version and corresponded to 30 patients on placebo. Data given are mean +/- SD. Wilcoxon test for matched pairs was used within groups and Mann-Whitney when comparing placebo and active treatment. A p level less than 0.050 was regarded as statistically significant.
Results: Treatment with the high seed level resulted in a statistically significant reduction in chemotaxis from 28.6 +/- 13.5 to 5.6 +/- 3.4 in a group of eight animals ($p<0.010$). A modest and insignificant increase was observed in a placebo group of identical magnitude, $p<0.050$ comparing the two groups. The low dose seed preparation, when tested in another eight animals resulted in an insignificant 3% increase of chemotaxis ($p<0.830$). No change in chemotaxis was observed in the placebo group and there were no significant difference between groups. Patients reaction to the high seed and low seed preparation supported the observations from animal studies. Treated with the A version (1.2 g seeds daily) resulted in a insignificant CRP reduction from 2.07 +/- 2.19 to 1.68 +/- 1.28 mg/l, a reduction of 18%. In the placebo group CRP levels increased statistically significant from 1,43 +/- 0.91 to 1.89 +/- 1.35 mg/l ($p<0.014$) an increase which is most likely due to reported seasonal variation. The two groups were significantly different from each other as indicated by a Mann-Whitney p value of 0.042. In the B group receiving low dose seed treatment (0.2 g daily) there was a modest and insignificant increase of 20% in CRP levels, which was not significantly different when compared to observations from the corresponding placebo group ($p<0.339$).
Conclusion: The present data suggest that Rose-hip seeds are of importance to keep the optimal anti-inflammatory profile in Rose-hip preparations.

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AN ORAL PREPARATION CONTAINING HYLAURONIC ACID (ORALVISC®) CAN NORMALIZE THE TURNOVER OF HYALURONIC ACID IN SYNOVIAL FLUID OF OSTEOARTHRITIC KNEE PATIENTS

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Purpose: Osteoarthritis (OA) is a degenerative joint disease in which articular cartilage matrix is no longer in homeostatic balance, resulting in a net loss of chondroitin sulfate (CS)-rich glycosaminoglycans (GAGs). The pro-inflammatory environment of synovial fluid has been shown to result in an increase in hyaluronic acid (HA) turnover. Consequently the turnover of CS and HA are considered key parameters to evaluate the degree and evolution of OA. We conducted a double blind randomized clinical trial to determine if there would be changes in turnover of CS and HA in the synovial fluid (SF) of knee OA patients treated with a patented hyaluronic acid formulation for oral use (Oralvisc®) as compared to placebo.
Methods: 51 symptomatic knee OA patients were recruited sequentially at the time of an outpatient visit for OA. Subjects were between the ages of 50-75 years, had knee effusion, and a pain visual analog score (VAS) >50mm. 40 patients completed the study, 21 had been randomly selected to receive 80 mg daily of Oralvisc®, and the remaining 19 had identical appearing placebos. Each month they were evaluated for VAS and WOMAC pain and joint function. A subset of 10 subjects per treatment group began a 2-day long oral administration of heavy water (35 mL ^2H_2O TID for 2 days) at the conclusion of the treatment phase (Week 12), followed by a synovial fluid aspiration in the affected knee. Synovial fluid lavage samples were analyzed for ^2H-labeling of component GAGs by gas chromatography/ mass spectrometry (GC/MS) to obtain fractional synthesis rates of HA and CS in SF.
Results: The age, sex, race, BMI, KL scores, as well as VAS pain and WOMAC function were balanced between groups at the beginning of the trial. Treatment with oral HA during 3 months resulted in a significant improvement in VAS pain ($p=0.0035$), WOMAC pain ($p=0.0259$) and WOMAC function ($p=0.0132$) compared to placebo. The stable-isotope-mass spectrometry method was successfully implemented for the clinical study of SF GAG kinetics. The rate of HA turnover was 0.78±0.42 / day (i.e., 78% per day) in placebo-treated OA patients ($n=10$; Fig. 1). With 12 weeks of oral HA treatment, the mean rate of HA turnover declined by 45% to 0.42±0.24 / day ($p = 0.046$). While we have not directly compared the rate of HA turnover in normal vs. osteoarthritic knees, comparison of the current results with data from a previous study in ACL patients suggests that these osteoarthritis patients (0.78 / d) have elevated HA turnover relative to "normal" subjects (0.25 / d), and that oral HA partially normalized the HA turnover rate. CS molecules in the SF were also highly enriched, with the majority of the patients recording the maximum measureable turnover rate for this assay (>140% / day). Thus, virtually all of the SF-derived CS was recently synthesized, suggesting a defect in the retention of potential repair molecules in osteoarthritic cartilage.
Conclusions: This is the first study of its kind to show that the use of an oral HA (Oralvisc®) agent in knee OA patients can lead to a significant impact on HA turnover in synovial fluid. The normalization in HA turnover was paralleled by an overall decrease in pain scores and improvement in joint function. Further studies are needed to investigate its mechanism but this novel form of oral HA may provide a safe alternative treatment to correct the homeostatic imbalances in the progression of OA.

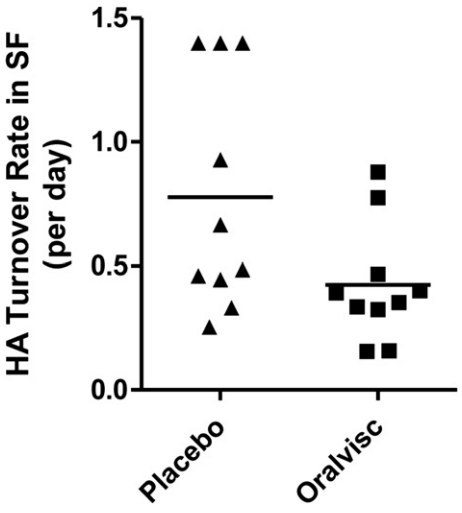

Figure 1. Turnover rate of HA in the knee synovial fluid of osteoarthritic patients. Bar shows the group mean (ANOVA $p = 0.046$).

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SULFORAPHANE REPRESSES MATRIX-DEGRADING PROTEASES AND PROTECTS CARTILAGE FROM DESTRUCTION IN VITRO AND IN VIVO

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Purpose: Broccoli is rich in glucoraphanin (a glucosinolate). When broccoli is cut or chewed, glucoraphanin is converted by an enzyme, myrosinase, to sulforaphane (an isothiocyanate). Similar compounds are derived from other cruciferous vegetables and these compounds are therefore accessible via the diet. Sulforaphane (SFN) has been reported to regulate signalling pathways relevant to chronic diseases. Our study investigated whether sulforaphane can abrogate cartilage destruction in osteoarthritis and examined mechanism of action in chondrocytes.
Methods: The bovine nasal cartilage explant model (BNC) and destabilisation of medial meniscus (DMM) murine model of osteoarthritis were used to study chondroprotection by SFN. Histone acetylation, gene expression, transcription factors nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor kappaB (NF-κB) signalling were examined.

Results: SFN abrogates cytokine-induced destruction of bovine nasal cartilage at the level of both proteoglycan and collagen breakdown (10μM compared to cytokines alone). It also decreases arthritis score in the DMM murine model of osteoarthritis (3μmol daily dose SFN in diet versus control chow). SFN inhibited cytokine-induced metalloproteinase expression in primary human articular chondrocytes (HACs) and in fibroblast-like synovial cells (FLS). SFN acts independently of the Nrf2 transcription factor and histone deacetylase activity in HACs, but does mediate prolonged activation of Jun kinase (JNK) and p38 MAP kinase. SFN attenuates NF-κB signalling through at least inhibition of DNA binding in HACs with attenuation of expression of several NF-κB dependent genes.
Conclusions: SFN, at levels which can be obtained through a high broccoli diet, inhibits the expression of key metalloproteinases implicated in osteoarthritis independently of Nrf2 and blocks inflammation at the level of NF-κB to protect against cartilage destruction in vitro and in vivo. Future studies in man will ascertain the potential of this compound in human osteoarthritis.

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COLLAGEN METABOLISM OF HUMAN OSTEOARTHRITIC CARTILAGE AS MODULATED BY COLLAGEN HYDROLYSATES

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Purpose: Collagen hydrolysates (CHs) are mixtures of collagen peptides and are popular nutraceuticals used for prophylaxis of osteoarthritis (OA). Collagen type I hydrolysates were found to stimulate the synthesis of proteoglycans and collagen by cultured healthy bovine articular chondrocytes [Oesser et al. 2003]. Recently a ligand-receptor interaction of small collagen fragments with integrin domains were found which might serve as a molecular mode of action of CHs [Siebert et al. 2010; Stoetzel et al. 2012].
The aim of our study was to determine for the first time whether and to what extent two commercially available CHs modulate 1) the synthesis of collagen from human articular OA cartilage, and 2) the degradation of collagen and proteoglycans from human OA cartilage obtained from human knee joints.
Methods: CHs from porcine (Mobiforte®, Astrid Twardy) and fish (FGH, Peptan™ F 5000, Rousselot) origin were used for our experiments. Peptides from CHs were chemically characterized using MALDI-TOF mass spectrometry, Atom Force Microscopy (AFM) as well as Nuclear Magnetic Resonance (NMR) spectroscopy.
The degree of OA changes was estimated according to Collins. In order to determine the collagen synthesis, explants were radiolabeled with [3H]-proline, washed several times, and radiolabeled again with [14C]-proline in the presence of 0-10 mg/ml CHs [Goodwin et al. 2008]. Proteoglycans were determined by the DMMB-method, NO by the Griess reaction, whereas MMP-1,-3,-13 and collagen type II within media were measured using ELISA kits. Cell viability was evaluated microscopically using fluorescein diacetate and propidium iodide. Untreated explants served as controls. Data presented are mean ± SD (n=6). Groups of data were evaluated using ANOVA and the Friedman test. Significance was set to $p < 0.05$.
Results: MALDI-TOF and 2D-TOCSY NMR analysis revealed qualitative differences between both CHs with respect to peptides identified in each preparation, width of molecular weight distribution and the average molecular weight.
Peptan™ F 5000 and Mobiforte® did not modulate the collagen synthesis. Only Mobiforte® induced an increased loss of proteoglycans from explants into nutrient media, whereas no loss of collagen was observed. Significantly elevated levels of NO, MMP-1, -3, -13 and/or PGE2 were found for FGH and Mobiforte®. CHs are not cytotoxic even when tested at a high concentration of 10 mg/ml.
Conclusion: Our investigation shows for the first time that CH preparations differ with respect to both the composition of peptides and their biological activities on human chondrocytes. Thus, their biomedical properties have to be studied thoroughly both in vitro and in animal as well as clinical trials before being applied as safe and effective nutraceuticals in patients.

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PAIN HAS A STRONGER ASSOCIATION WITH THIGH MUSCLE STRENGTH THAN THE RADIOGRAPHIC STAGE OF KNEE OSTEOARTHRITIS - DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: Previous studies reported an association between reduced (quadriceps) muscle strength and knee osteoarthritis (OA), but it remains unclear to what extent this relationship depends on structural (radiographic, i.e. rOA) status or pain. To disentangle the above relation, we here study extensor and flexor muscle strength across participants with different radiographic OA stages, with and without pain, in a large cohort of subjects with or at risk of knee OA.
Methods: Participants were drawn from the entire baseline incidence and progression subcohort cohort of the Osteoarthritis Initiative (OAI; n=4674), including all right knees with central Kellgren-Lawrence grade (KLG) readings and complete information on the WOMAC pain score and measures of isometric muscle strength (n=3809). Of these, 1378 were KLG0, 698 KLG1, 1054 KLG2, and 679 KLG3/4. In each KL stratum, observations were stratified between WOMAC knee pain scores: 0 (no pain), 1-5, and >5 (severe pain) [range 0-20], as the mean was close to 5 in women and men. Measurements for maximum isometric extensor and flexor strength at 60° knee flexion were taken from the OAI database ("Good Strength Chair", Metitur Oy, Jyvaskyla, Finland) and to account for inter-subject differences these were normalized to body weight (strength/weight). Of the OAI healthy reference cohort (KLG0, asymptomatic, and without OA risk factors), only a small subset of (right) knees had strength measurements availabale (13 women, 12 men), which were used for comparison. Separate slopes ANCOVA models, with age as covariate and contrast analyses (for age-adjusted means), were used to compare: a) limbs with severe vs. no pain within each KLG stratum b) painless limbs across KLG strata, and c) painless limbs with rOA (KLG2-4) vs. healthy reference limbs.
Results: In both men and women, and across all KL grades, extensor and flexor strength/weight were significantly and substantially less in severely painful (WOMAC>5) than in painless limbs (Fig. 1); only flexor strength/weight in male KLG3/4 knees did not reach statistical significance (p=0.08). In painless female limbs, extensor and flexor strength/weight differed significantly between KL strata (p=0.046/0.02). In male limbs, extensor (but not flexor) strength/weight differed between KL strata (p<0.001). Although strength tended to be lower with higher KL grades, painless KLG3/4 limbs displayed similar to greater strength than KLG 0/1 limbs with severe pain (Fig.1). In women, extensor strength/weight was by 10% [95%CI -19%,+39%) lower in painless rOA than in healthy reference knees (n=303 vs. 13); in men extensor strength/weight was by 11% [95%CI -4%,+25%] lower in painless rOA than in healthy reference knees (n=238 vs. 12). However, these differences did not reach statistical significance (p=0.50/0.15) and similar results were found for flexor strength/weight (p=0.06/0.58).
Conclusion: Knee pain intensity is observed to be a very strong determinant of muscle strength in men and women. A weak association between radiographic stage (KLG) and muscle strength in painless limbs is seen, particularly in women, but less so for flexor strength in men. Hence, pain appears to have a much stronger association with thigh (and specifically quadriceps) muscle strength in knee OA than the radiographic disease stage. Longitudinal studies need to show whether

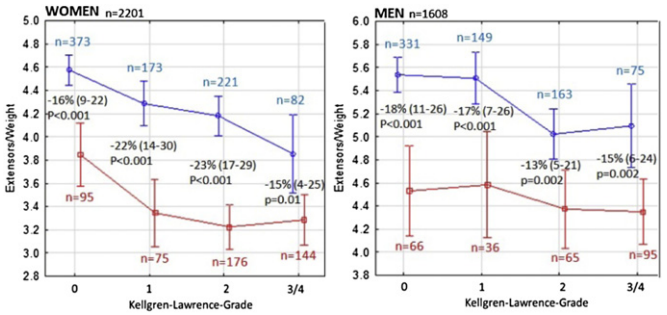

Fig 1. Extensor strength/weight in women (left) and men (right) without pain (WOMAC = 0, gray line) and those with severe pain (WOMAC>5, black line). Percent differences are shown with 95% confidence intervals.